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How can nanoparticles be used in sentinel node detection?

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Sentinel node biopsy (SNB) is the standard of care for axillary staging in breast cancer patients with a clinically and radiologically normal axilla. The concept is based upon the exclusion of metastases in the first draining lymph node(s) from the primary tumour. The current practice utilises a radiolabelled tracer and blue dye (combined technique) with reported sentinel node identification rates of 96.4 per cent, and a false negative rate of 7.3 per cent in a large meta-analysis. [1] Although successful, this technique is limited by its dependence upon radioisotopes - with their inherent strict requirements for handling, transport, disposal, and close access to nuclear medicine facilities. Blue dye is also associated with prolonged skin staining and reports of anaphylaxis in 0.9 per cent of patients. [2] These limitations have resulted in the on-going development of alternative techniques for SNB. [3, 4]

One of the most promising radioisotope independent techniques for SNB comprises an interstitial injection of superparamagnetic iron oxide (SPIO) nanoparticles, which are identified intra-operatively with a handheld magnetometer and through visual brown-black staining of nodes. The magnetic technique has now been evaluated in a number of studies and confirmed on meta-analysis to be non-inferior to the radioisotope-dependent technique in breast cancer - with sentinel node identification rates of 97.1 and 96.8 per cent respectively [3] – and demonstrated technical benefits over other novel techniques. [4] The non-inferiority of the magnetic technique has

also been demonstrated in the staging of malignant melanoma [5] and has been applied to a range of solid cancers in small cohort series, which has demonstrated its feasibility in the staging of these malignancies. [6-8] What is still lacking is randomised controlled trial evidence, before the magnetic technique can be considered as an alternative to the standard technique.

The current trend within axillary management in breast cancer is towards quantification of the axillary metastatic burden. The identification of low axillary burden has been shown to allow avoidance of formal axillary node clearance (ANC), without compromising overall and disease-free survival at long-term follow-up. [9] This evidence is leading to the concept of 'selective axillary surgery', which would allow targeted excision of only metastatically involved nodes. This relies upon improved pre-operative imaging combined with novel techniques to guide surgeons intra-operatively, to involved nodes. Magnetic nanoparticles have been used for axillary staging non-invasively with magnetic resonance imaging (MRI) and demonstrated a sensitivity and specificity for the detection of axillary involvement of 98 and 96 per cent respectively. [10] A limitation to implementation of this approach is the elevated false positive rate compared to SNB. [11] This has led to the development of dual probes to allow for the benefits of multi-modal imaging. Magnetic tracers have been conjugated with radiolabelled bisphosphonates in order to allow simultaneous, excellent anatomical visualization on MRI and computerized tomography (CT) scanning, in addition to functional categorization on positron-emission tomography (PET). [12] Triple-modal imaging magnetic nanocapsules, encapsulating hydrophobic SPIO to magnetically target solid tumours after intravenous administration has been developed. [13] Within a murine model the magnetic polymeric nanocapsules demonstrated a 2-fold increase in tumour uptake when a static magnetic field was applied. [13] The probes allowed fluorescence, MRI and nuclear imaging to be performed, providing complementary information on the spatial distribution of the nanocarrier within the

tumour. Consequently, multi-modal probes, which target metastatic foci have been developed. These have included fluorescent-ferrite beads containing both magnetic iron oxide and fluorescent europium complexes, which have been labeled with antibodies to a range of clinically relevant antigens (BNP, PSA, EGFR, CA19-9 and HB-8509). The activation of these particles by application of a magnetic field allows for immunohistochemical staining and enhanced intensity of fluorescence and focal quantification of antigen, resulting in targeted therapies and diagnostics. [14]

The use of multimodal probes allows for optimization of pre-operative imaging to characterize lymph nodes and quantify nodal burden. The multimodal labelling also allows the surgeon to utilise different techniques to localise involved nodes and target them precisely. Open surgery could be directed with a handheld magnetometer to identify 'hot spots' of magnetic tracer accumulation and hence metastatically involved nodes. Minimally invasive, percutaneous techniques could be used to target the magnetic tracer accumulation (nodes) under MRI-guided vision and excise specimens en bloc. Alternatively, completely, non-invasive techniques using MRI-guided high intensity focused ultrasound (HIFU) could be applied to the same targets of focal iron accumulation and ablate the specimens without excision. This could equally be potentially achieved by the application of an alternating magnetic field to induce targeted magnetic hyperthermia.

It has been demonstrated from pre-clinical porcine models [15] that only 2 per cent of injected SPIO reaches the draining sentinel nodes when the clinical volume of 2 mL (54 mg iron oxide) of magnetic tracer is injected interstitially. Consequently, evidence has emerged from the largest trial of the magnetic technique that this retention of iron can produce void artefacts on MRI and that in some patients this can be prolonged. [16] This area requires prospective evaluation to confirm these

findings but it is essential that on-going research attempt to reduce iron retention at injection sites. The injection of lower volumes (0.5 mL) intra-tumorally was explored clinically but resulted in low sentinel node identification rates compared to the standard combined technique (85 *versus* 97 per cent). [17] Porcine models demonstrated a significant inverse relationship between increasing injection volumes and percentage iron-uptake (relative to injected dose) by sentinel nodes (P<0.001). [15] Future work is needed to evaluate lower volume interstitial injections and the feasibility of intravenous administration.

The impact of increasingly conservative axillary management in breast cancer has increased the importance of pre-operative staging using ultrasound. Trials are assessing whether a negative axilla on ultrasound may eliminate the need for SNB completely. It is very likely that in future, pending the results of on-going trials, that the clinical indication for SNB may be reduced. However, accurate staging will still be required in those patients who possess an abnormal pre-operative axillary ultrasound. This will be particularly the case in patients who are receiving primary systemic therapy and who will be assessed for their response before undergoing definitive surgery. It has been shown that when this cohort of patients undergoes SNB, they have an elevated false negative rate (12 per cent), which is lower on removal of 3 or more nodes (9 per cent). [18]If the suspicious nodes are clipped at ultrasound and removed at SNB, then the false negative rate falls to less than 2 per cent. [19] The application of a solid ferromagnetic marker – Introduced [19, 20] into the suspicious nodes - which could subsequently be excised under intra-operative guidance of a handheld magnetometer, would be clinically relevant and avoid technical and logistical difficulties associated with radioactive markers or wires. Both removal of more nodes and clipping of nodes have not been confirmed in subsequent clinical trials as yet.

The use of SNB is in an advanced stage within breast cancer compared to other solid cancers. This has resulted in the emergence of the necessity to quantify axillary lymph node burden in determining management rather than simply confirming the presence or absence of metastatic involvement. This places nanomedicine in a unique position to allow the emergence of targeted imaging and therapies. These therapies will allow the development of minimally invasive, selective axillary surgery, targeting metastatic nodes alone and provide more personalized treatments for cancer patients of the future.

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